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Research Article

MOLECULAR CHARACTERIZATION OF ANTHOCYANIN SYNTHASE GENE OF PURPLE AND WHITE FLESHED VARIANCE OF *DIOSCOREA ALATA*

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ABSTRACT

In the present study, Molecular analysis of Anthocyanin synthase gene (ANS) study was carried out at ICAR-CTCRI of Greater yam (*Dioscorea alata*) tuber (white and purple fleshed).The DNA obtained from greater yam tuber was quantified showing good purity of DNA. Using primer ANS DA, brighter bands of DNA of Greater yam tuber with product size 210bps was obtained. After sequencing, data analysis was done, showing 96% similarities through BLAST software between the variety tubers of Greater yam. The results were valuable for showing, high amount of Anthocyanin present in Greater yam tuber with equality between Greater yam tubers.

Keywords: Anthocyanin synthase gene, *Dioscorea alata,* ANS DA, BLAST.

INTRODUCTION

Tubers are distended structures in some plants used as storage organs for nutrients. They are used for the plant's perennation (survival of the winter or dry months), to provide energy and nutrients for regrowth during the next growing season, and as a means of asexual reproduction. Stem tubers are formed from rhizomes or stolons. The Greater yam (*Dioscorea alata*L.) has the following known benefits: toning the kidneys, nourishing the stomach, refreshing and enriching the saliva, and aiding the lungs. Therefore, it can be used as both a medicinal and edible plant and it is widely cultured in South Pacific and southern China (Champagne *et al*. 2011).

Anthocyanins are the important plant pigments used for colouring of plant organs. They act as antioxidants, phytoalexins, or as antibacterial agents. Anthocyanins are water-soluble vacuolar pigments that gives red, purple, or blue to the plant. They belong to a class of secondary metabolites namely flavonoids which are synthesized via the phenylpropanoid pathway; they are odourless and nearly flavourless, contributing to taste as a moderately astringent sensation. Anthocyanins are present in the tissues of leaves, stems, roots, flowers, and fruits. Anthocyanins are derived from anthocyanidins by adding sugars (Andersen and Øyvind M, 2001).

In greater yam (white and purple fleshed) tuber the anthocyanins are acylatedcyanidin and peonidin type showing Anticancer and Antioxidative activity. Anthocyanin synthase (ANS) is an enzyme which belongs to the family of oxidoreductases. In the present study molecular analysis of Anthocyanin synthase gene and comparison of Anthocyanin synthase gene was done between Greater Yam varieties.

MATERIAL AND METHODS

Collection of Plant

Greater yam (*Dioscorea alata*) tuber were collected from experimental field farm of ICAR Central Tuber Crops Research Institute, Thiruvananthapuram which was Greater yam (white and purple) fleshed tuber. The collected samples were then taken to the laboratory and wiped with 70% ethanol for surface sterilization and further the samples were used for DNA extraction.

DNA Extraction

DNA isolation was done using CTAB method with some modification (Porebski *et al*., 1997). Agarose gel electrophoresis was done to check the quality of DNA where the bands

were visualized. After electrophoresis gel was placed in the gel document unit and bands were visualized. The gel image was stored in the computer. The separated fragment in each lane was viewed (Goldmann*et al.,* 2001).

Quantitative Real-Time PCR

DNA Quantification was carried out by UV Spectrophotometer (Systronics). The Equipment was calibrated with distilled water as blank. 10µl of DNA sample was added to the quartz cuvette and made up the volume to 1ml with distilled water. The absorbances of the solution were taken at wavelengths of 260 and 280nm. The ratio A260/ A280 was calculated and the DNA concentration calculated using the relationships for double stranded DNA (Bulyk*et al*., 1999).The Real-time PCR was carried out by using ANS primer gene sequence of greater yam of both Forward and reverse sequence ANS F: 5'-TGGCCTGCA GGTCTTCTACGA-3'and R: AACGGACGCAGCAGCACCTT using Thermal cycler (BioRad C1000TM Thermal Cycler) under the conditions 94◦C 5 m followed by 30 cycles at 94◦C 1 m, 50◦C 45 s, 72◦C 1m 30s, 72◦C 8 m with 4◦C hold.

Sequence Data Analysis

BioEdit is a sequence alignment editor written for various versions of Windows. It is a Software with convenient features that makes alignment and manipulation of sequences relatively easy. Several sequence manipulation and analysis options allows to view and manipulate sequences with simple point-and-click operations.

The sequence data analysis was done by using BioEdit software. In this software the sample sequence of *Dioscorea alata* was imported. In the BioEdit software, the forward sequence was imported into this software and further the reverse sequence was clicked and nucleic acid was selected after which the reverse complement was done of the reverse sequence and imported in this software down to forward sequence. After importing the reverse sequence, the sequence was then aligned in Clustal W.

RESULT AND DISCUSSION

DNA Extraction

Previous study reported that Kang *et al*. (1998) isolated good quality of DNA from Greater yam leaves. Here DNA was isolated from greater yam using CTAB (Cetyl Trimethyl Ammonium Bromide) method. The quantity and quality of DNA was tested by spectrophotometer and agarose gel electrophoresis respectively to ensure the use of good quality DNA.

Quantification of DNA

Previous study Brunner *et al* reported that good quantity of DNA was obtained from leaf varieties of Greater yam. In this study, isolated DNA of greater yam was quantified using the Nanodrop (DeNovix). NanoDrop method determines absorbance at 260nm by taking reading at wavelength of 260 nm. This method not only quantifies the DNA but also checks the purity of DNA in the sample. Based on this method, the isolated DNA showed good purity (1.8) and quantity (250-1120) (Table 1). Here the concentration and purity was found high in *Dioscorea* white fleshed tuber than purple fleshed tuber.

Polymerase Chain Reaction (PCR)

The PCR product loaded in 2% gel was electrophoresed and further the gel was placed in the gel document unit and bands were visualized. The gel image was stored in the computer. The separated fragment in each lane was viewed (Fig 1).

Through PCR it was observed that the genomic DNA bands had an expected size range of greater yam (210bp) using ANS primer (Fig 1).

Sequence Data Analysis

Previous studies reported that the genetic variability in *D. alata* (Greater yam) is dominantly caused by natural gene flow rather than vegetative reproduction and by a natural hybrid between species of *Dioscorea* (Lebot*et al*., 1998). In this study as brighter band of greater yam was obtained from PCR. DNA sample of greater yam was selected for cycle sequencing. After sequencing, the sequence data analysis was done by using BioEdit software.

In this software the forward and reverse sequence of *Dioscorea alata* white and *Dioscorea alata* purple fleshed tuber was imported. After importing the sequence of Greater yam tuber, the sequence was then aligned in CLUSTAL W. The sequence aligned in CLUSTAL W was run on BLAST. The sequence of *Dioscorea alata* between white and purple fleshed showed 48/50 identity with greater similarity (96%) (Fig.2).

CONCLUSION

The results were valuable for showing, high amount of Anthocyanin present in Greater yam tuber with 96% similarities between the variety tubers of Greater yam which will be helpful in treating various diseases.

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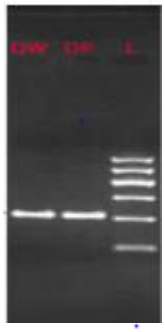
Table 1: Quantification of DNA

Sample	Concentration	A260/230	A260/280
DW	988.15	1.8	1.8
DP	499.5	1.81	1.84



Dioscorea alata Anthocyanin synthase (ANS) mRNA, complete cds

Sequence ID: gb|KP729182.1|Length: 1320Number of Matches: 1

Range 1: 918 to 967 GenBank Graphics Next Match Previous Match

Alignment statistics for match #1

Score Expect Identities Gaps Strand

Fig. 1: PCR product amplified

75.2 bits(82) 2e-10 48/50(96%) 2/50(4%) Plus/Plus

Query 45 CTGTACAAGAG-GTGCTCCATCGTGGGCTTG-TAATAAGGAGAAGGTGAG 92

Sbjct 918 CTGTACAAGAGTGTGCTCCATCGTGGGCTTGTTAATAAGGAGAAGGTGAG 967

Fig. 2: Sequence of white and purple fleshed greater yam imported in NCBI BLAST showed 96% similarity between them

REFERENCES

1. Andersen and Øyvind M. Anthocyanins. Encyclopedia of Life Sciences. eLS. John Wiley & Sons, Ltd. doi:10.1038/npg.els.0001909. ISBN 0470016175.
2. Bulyk ML, Gentalen E, Lockhart DJ and Church GM. Quantifying DNA protein interactions by double-stranded DNA arrays. Nature biotechnology. 1999;17(6).
3. Goldmann T, Zyzik A, Loeschke S, Lindsay W and Vollmer E. Cost-effective gel documentation using a web-cam. Journal of biochemical and biophysical methods. 2001;50(1):91-95.
4. Kang DK., Kim SK, Min GG, Chung SH, Lee SP and Choi BS. Study on

development of Sanyakju. Traditional folk wine made of chinese yam (Dioscoreabatatasdecne). RDA Journal of Industrial Crop Science (Korea Republic). 1998.

5. Lebot V, Trilles B, Noyer JL and Modesto J. Genetic relationships between Dioscoreaalata L. cultivars. Genetic Resources and Crop Evolution. 1998;45(6):499-509.
6. Porebski S, Bailey LG and Baum BR. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant molecular biology reporter. 1997;15(1):8-15.
7. Yin JM, Yan RX, Zhang PT, Han XY and Wang L. Anthocyanin accumulation rate and the biosynthesis related gene expression in Dioscoreaalata. Biologiaplantarum. 2015;59(2):325-330.